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CONVENTION

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NOTICE OF ENTITLEMENT

We, **REGENERON PHARMACEUTICALS, INC.** of 777 Old Saw Mill River Road, Tarrytown, New York 10591, United States of America state the following in connection with Australian Application No. 46735/93:

1. We are the nominated person.
2. The nominated person is the assignee of the actual inventors.
3. The nominated person is the assignee of the applicants of the basic application listed in the declaration under Article 8 of the PCT.
4. The basic application is the application first made in a Convention country in respect of the invention.

Dated: 22 December 1994

By **PHILLIPS ORMONDE & FITZPATRICK**
Patent Attorneys for the Applicant
By:

David B Fitzpatrick

To: The Commissioner of Patents

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ASSAY SYSTEM FOR DEGENERATIVE MUSCLE DISEASE

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(57) Claim

1. A method of detecting muscle denervation in a patient afflicted with such muscle denervation comprising:

- a) obtaining from said patient a sample of a body fluid;
- b) reacting said sample with a material which specifically binds to ciliary neurotrophic factor receptor alpha protein (CNTFR α) which is present in solution in said sample to provide a complex of CNTFR α and said material;
- c) determining a level of CNTFR α in said sample based on a measurement of said complex;
- d) comparing the level of CNTFR α determined in said sample to levels of soluble CNTFR α determined in samples of the body fluid of unaffected individuals, wherein an elevated level of CNTFR α in the sample is indicative of muscle denervation in the patient.

CORRECTED
VERSION *

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
pages 1/2-2/2, drawings, replaced by new page 1/1, due to
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(54) Title: ASSAY SYSTEM FOR DEGENERATIVE MUSCLE DISEASE			
(57) Abstract An assay system useful for detecting denervated or degenerating muscle by measuring the amount of soluble ciliary neurotrophic factor receptor in body fluids is described.			

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Assay System For Degenerative Muscle Disease

Introduction

5 The present invention relates to a diagnostic technique useful for detecting, diagnosing or monitoring neurological and/or muscular diseases, disorders or injuries. The invention provides for an assay system useful for detecting denervated or degenerating muscle by measuring the amount of soluble ciliary neurotrophic factor receptor (CNTFR α) in body fluids.

Background of the Invention

15 Ciliary neurotrophic factor (CNTF) is a protein that is required for the survival of embryonic chick ciliary ganglion neurons in vitro (Manthorpe et al., 1980, J. Neurochem. 34:69-75). The ciliary ganglion is anatomically located within the orbital cavity, lying between the lateral rectus and the sheath of the optic nerve; it receives parasympathetic nerve fibers from the oculomotor nerve which innervates the ciliary muscle and sphincter pupillae.

20 Over the past decade, a number of biological effects have been ascribed to CNTF in addition to its ability to support the survival of ciliary ganglion neurons. CNTF is believed to induce the differentiation of bipotential glial progenitor cells in the perinatal rat optic nerve and brain (Hughes et al., 1988, Nature 335:70-73). Furthermore, it has been observed to promote the survival of
25 embryonic chick dorsal root ganglion sensory neurons (Skaper and Varon, 1986, Brain Res. 389:39-46). In addition, CNTF supports the survival and differentiation of motor neurons and hippocampal neurons. (International Application No. PCT/US 90/05241)

Recently, CNTF has been cloned and synthesized in bacterial expression systems, as described in copending U.S. application No. 07/570,651, entitled "Ciliary Neurotrophic Factor," filed August 20, 1990 by Sendtner et al. incorporated by reference in its entirety herein.

In addition to CNTF, the receptor for CNTF (originally termed "CNTFR") has been cloned, sequenced and expressed (see copending U.S. application No. 07/700,677, entitled "The Ciliary Neurotrophic Factor Receptor," filed May 15, 1991^{and now abandoned} by Davis, et al. and International Application No. PCT/US91/03896, filed June 3, 1991 which are incorporated by reference in their entirety herein).

Unlike other known growth factor receptors which have an extracellular domain, a hydrophobic transmembrane domain and a cytoplasmic domain, the CNTF receptor does not appear to have a cytoplasmic domain. Furthermore, it is linked to the cell surface via a covalent linkage from the protein to an oligosaccharide which in turn glycosidically linked to phosphatidylinositol (referred to as a "GPI-linkage"). GPI linkages play a role in the attachment of proteins, such as alkaline phosphatase (APase) to membranes. The role of GPI linkages in the function of biological membrane components have been elucidated as a major means of anchoring proteins to biological membranes in the case of at least 30 distinct proteins. [(Slein, et al., J. Bacteriol. 80:77 (1960); Low, et al., Biochemistry 19:3913 (1980)].

Treatment of the cell-surface membrane of CNTF responsive cells with phosphatidylinositol-specific phospholipase C (PI-PLC) releases CNTFR from the cell membrane. Such release is prohibited,



however, if CNTF is first bound to the receptor prior to exposure to the enzyme, indicating a possible interaction between CNTF, its receptor and a third, signal transducing component.

5 The discovery that CNTFR and CNTF may form a complex that interacts with a membrane bound, signal transducing component suggested therapeutic activity of a soluble CNTF/CNTFR receptor complex. In copending application U.S. Serial No. 801,562 filed December 2, 1991 by Yancopoulos, et al. entitled "Cell Free Ciliary Neurotrophic Factor/Receptor Complex", which is incorporated by
10 reference in its entirety herein, CNTF and CNTFR are combined to form a stable, biologically active complex that can be used as a differentiation or proliferation factor in cell types that express signal transducing receptor components belonging to the CNTF/IL-6/LIF receptor family.

15 One such signal transducing component involved in high affinity binding of CNTF and the subsequent functional response of the cell has been identified as gp130, a β component common to the IL-6, Oncostatin M, LIF family of receptors. A further β component identified as being involved in binding and signal transduction in
20 response to LIF (LIFR β) appears to be the same or similar to a β component necessary for response to CNTF. (As a consequence of the identification of β components necessary for binding and signal transduction of CNTF, what was originally generally termed CNTFR is currently referred to as CNTFR α). Accordingly, as described in
25 U.S. application Serial No. 07/865,878 filed on April 8, 1992 and now
abandoned
^ entitled "Cell-Free Ciliary Neurotrophic Factor/Receptor Complex", which is incorporated by reference in its entirety herein, signal transduction can be initiated by treatment of cells expressing both



gp130 and LIFR β with a soluble CNTF/CNTFR α complex.

Alternatively, target cells not previously responsive to CNTF, but expressing LIFR β and gp130 (such as LIF-responsive cells) can be made responsive to CNTF by attaching the CNTFR α to the cells and subsequently treating with CNTF.

The molecular cloning of the coding region for human CNTFR α (hCNTFR α) enabled the generation of probes useful for detecting the presence of receptor message in a variety of tissues. The results of such studies indicated that CNTFR α mRNA was detectable in tissues of the central nervous system, sciatic nerve, adrenal tissue and in skeletal muscle. As described in copending application U.S. Serial No. 07/700,677^{now abandoned}, entitled "CNTF Receptor" which is incorporated by reference in its entirety herein, a detailed analysis of the CNTF receptor expression in muscle indicated that the CNTF receptor is expressed in both myotube and myoblast muscle cell lines of either mouse or rat origin, as well as in both red slow-twitch soleus muscle and white fast-twitch extensor digitorum longus (EDL) muscle of the rat.

Further described in U.S. application 07/700,677^{now abandoned} is the finding that CNTF receptor mRNA was increased in both soleus and EDL muscle in animals that were first denervated for 72 hours relative to their sham-operated contralateral controls.

Brief Description of the Drawings

Figure 1. CNTFR α levels in plasma increase after nerve crush in rats. Plasma (200 μ l) from various post-surgical timepoints was extracted with biotinylated CNTF immobilized on streptavidin conjugated to agarose beads as described in the Example section.



The figure shows an immunoblot developed with secondary antisera conjugated to alkaline phosphatase. Samples are 25 fmoles CNTFR α standard (lane 1), PBS spiked with 25 fmoles CNTFR α and extracted with immobilized CNTF as control (lane 2), rat plasma samples taken at the indicated time after nerve crush: 0 h (lane 3), 6 h (lane 4), 18 h (lane 5), 24 h (lane 6), 30 h (lane 7), 42 h (lane 8), 48 h (lane 9), 72 h (lane 10).

Figure 2. CNTFR α in urine from normal humans and patients with ALS. Urine samples (1 ml) were brought to neutral pH and incubated with biotinylated CNTF to extract CNTFR α as described in the Example section, then analyzed by immunoblotting. Lane 1 is 12.5 fmole of recombinant CNTFR α , and lane 2 is ALS urine spiked with 12.5 fmole of CNTFR α . Lanes 3-6 are urine samples from ALS patients while lanes 7-10 are urine samples from unaffected individuals.

Summary of the Invention

The present invention relates to methods of diagnosing, detecting or monitoring neurological and/or muscular injuries, disorders or diseases.

An object of the present invention is to diagnose injury or disorders or diseases of the muscle.

Another object of the invention is to diagnose injury or disorders or diseases associated with muscle denervation or degeneration.

A further object of the present invention is to monitor the progression of a neurological and/or muscular injury or disease or disorder.

Another object of the invention is to measure the effect of a particular treatment on a neurological or muscular disease or disorder.

5 These and other objects are achieved in accordance with the invention by measuring the amount of CNTFR α in a body fluid such as blood or urine.

Detailed Description of the Invention

10 The present invention relates to a method of diagnosing diseases and disorders in human or animals in which muscle degeneration plays a role. It is based on the finding that CNTFR α message increases in denervated muscle and the further discovery that, under such circumstances, the receptor is shed. As described herein, the presence of the shed receptor in body fluids provides a
15 means of monitoring or diagnosing nerve or muscle injury or degeneration by measuring the amount of soluble CNTFR α in body fluids.

Soluble cytokine receptors which bind IL-6 and IFN- γ have been reported in urine under normal physiological conditions [Novick, et
20 al. J. Exp. Med. 170 (1989):1409-1414]. In addition, truncated nerve growth factor receptor occurs in urine of neurologically normal subjects during normal development. [DiStefano, et al. Annals of Neurology 29:13020(1991)]. Levels of truncated nerve growth factor receptor in the urine have been suggested as a means of determining
25 the presence of nerve growth factor receptor bearing tumors [Johnson, E. U.S. Patent No. 4,800,241 (1989)]. There is no indication that these, or any other receptors, are shed in response to muscle or nerve injury or disease.

As described herein, applicants have discovered a dramatic increase in the level of message for CNTF receptor in soleus muscle after nerve transection or crush. As innervation of the muscle was reestablished (in the case of the sciatic nerve crush), the level of CNTF receptor mRNA decreased.

Despite the increase in CNTF receptor message after crush, the amount of CNTFR α protein in denervated muscle tissue did not appear to increase commensurately. In view of the readily cleavable GPI membrane anchor of CNTFR α , this suggested the possible release of the receptor into surrounding blood. Applicants subsequently discovered that the dramatic increase in the level of the CNTF receptor mRNA in response to nerve crush or injury appeared to be reflected by the presence of an elevated level of the encoded CNTF receptor in a body fluid.

According to the present invention, an elevated level of CNTFR α in body fluid such as blood, plasma, urine or cerebral spinal fluid of a patient relative to the level of such receptor in a normal subject is used as an indicator that the patient is afflicted with some form of muscle degeneration. Such degeneration may be caused by an injury, by a degenerative muscle disease or disorder, or by a disease, disorder or damage to the nervous system which results in denervation of muscle. Such diseases or disorders include, but are not limited to, degenerative or inflammatory muscle diseases such as muscular dystrophy, myotonic dystrophy, fascio-scapulo-humeral dystrophy, limb girdle dystrophy, distal muscular dystrophy or myositis or peripheral neuropathies associated with diabetic neuropathy, acute neurapraxia, neurotmesis or axotmesis. In addition, the methods described herein can be used to diagnose or

monitor neurological degenerative diseases, especially those associated with degeneration of motor neurons, such as amyotrophic lateral sclerosis, spinal muscular atrophy, post-polio syndrome, infantile muscular atrophy, poliomyelitis or Charlot-Marie Tooth
5 disease or inflammatory or demyelinating neurological diseases or disorders such as Guillain-Barré Syndrome or chronic inflammatory demyelinating polyneuropathy. The methods of the present invention may also be used to diagnose or monitor degeneration caused by nerve injuries such as those associated with carpal tunnel
10 syndrome, compression, mechanical severance of a nerve or a tumor. In addition, the methods disclosed herein may be utilized to diagnose neural or non-neuronal tumors.

The present invention contemplates not only diagnosis, but also monitoring of the progression of a neurological and/or muscular
15 disease or disorder, or the effect of a particular treatment on the progression of such a disease or disorder. In such cases, the level of CNTFR α in the body fluid is monitored over a set period of time. The efficacy of a particular treatment is determined by comparing the level of receptor in the body fluid of an afflicted individual
20 receiving treatment with the level of receptor in the body fluid of a comparable patient not receiving such treatment.

The level of CNTFR α in any body fluid of an afflicted patient may be measured and compared to the comparable level of such receptor in a normal patient. Preferably, the body fluid analyzed is
25 urine, blood or cerebral spinal fluid. Analysis of urine may require normalization to account for individual variation; one such method involves normalization to the urine level of creatinine, an established indicator of the variable water content between

individual samples [DiStefano, et al. Annals of Neurology 29: 130-20(1991)].

CNTFR α levels in plasma may be preferable in that normalization is generally not required. Purification and concentration may be accomplished by any number of methods known to those in the art; as described herein, a simple method of accomplishing such purification and concentration involves the use of biotinylated CNTF.

Analysis and quantitation of CNTFR α is exemplified herein by immunoblotting with an antibody raised and immunopurified against CNTFR α , such as, for example, antibodies raised against a peptide corresponding to the N-terminus of human CNTFR α . Alternatively, monoclonal antibodies, such as those produced using the method of Kohler and Milstein [Nature 256, 495-497 (1975)], may be used.

Immunoreactive proteins may be visualized using antibodies produced against the CNTFR α antibodies which are conjugated to an indicator such as alkaline phosphatase or ^{125}I , which enables quantitation of reactive bands. Other immunoassay procedures known to those in the art, such as radioimmunoassays or enzyme-linked immunosorbent assays (ELISA), can also be used in the method of the present invention.

Examples

EXAMPLE 1. Regulation of CNTF Receptor (CNTFR) mRNA in Deafferented Muscle

5 Significant levels of CNTFR mRNA can be detected in normal skeletal muscle. To determine whether this CNTFR mRNA is regulated in response to altered nerve/muscle interactions, the levels in muscle following sciatic nerve transection or crush were examined.

10

1.1 Methods

Experimental subjects and surgical methods were as follows: Sciatic nerves of adult male Sprague-Dawley rats were cut or crushed at the level of the sciatic notch. In nerve transection
15 experiments, nerves were transected and the distal stump turned back and tucked under overlying musculature. This procedure effectively prevented regeneration of the cut nerves. Nerve crush was produced by tightly compressing the sciatic nerve with a #5 jewelers forceps for ten seconds; this technique caused axons to
20 degenerate distal to the crush but allowed subsequent regeneration of the nerve. Soleus muscle was dissected from normal or operated rats at different times after transection or crush of the sciatic nerve. Total RNA was prepared by the lithium chloride precipitation method, and the CNTFR transcripts analyzed by immunoblotting with
25 an antibody (RG30) raised and immunopurified against a peptide corresponding to the N terminus of human CNTFR. A vector encoding the entire human CNTFR sequence was deposited under the terms of the Budapest Treaty with the Agricultural Research Service Culture

Collection in Peoria, Illinois on March 26, 1991 and assigned NRRL B-18789.

1.2 Results

5 One day following transection of the sciatic nerve, there was a striking increase in the level of CNTFR mRNA in soleus muscle. This increase was substantially attenuated 2 days after the lesion, but increased again slowly and progressively over 122 days. A dramatic increase in CNTFR mRNA also was observed one day following nerve
10 crush, followed by a relative decrease. A subsequent progressive increase in CNTFR mRNA levels was seen over the first post-operative week. However, in marked contrast to the transection condition in which regeneration of the nerve was prevented, the level of CNTFR mRNA in muscle denervated by crush stabilized in the
15 second post operative week and subsequently decreased as innervation of the muscle was re-established.

The normally high constitutive expression of CNTF receptor mRNA in the CNS was unaltered by trauma (data not shown).

20 In contrast to the increased level of CNTFR mRNA found after injury, no corresponding increase in the level of CNTFR protein was observed on the membrane surface. This suggested the possibility that receptor was released from the cell surface into surrounding body fluid.

EXAMPLE 2. Detection of CNTFR α in Body Fluid

2.1 Methods

5 2.1.1 Preparation of Biotinylated CNTF (bCNTF)

CNTF (150-400 mg in PBS, pH 7.4) was incubated with a 5-fold molar excess of NHS-LC-biotin (Pierce, typically dissolved to 5 mg/ml in PBS immediately before use) in a volume of 500 μ L or less for 2 hours at 4 C. The reaction was quenched by the addition of 50 μ L 3 M Tris, pH 8.2, for approximately 15 minutes.

10 Biotinylated mono and dimeric CNTF were purified and separated from excess biotinylation reagent by chromatography on a Superdex 75 HR 10/30 column (Pharmacia) using a Pharmacia FPLC system pumping PBS at a flow rate of 1 ml/min. Dimer and monomer bCNTF elute at times of 10 and 12 minutes respectively. Protein concentration and recovery were determined by optical density readings at 280 nm using an extinction coefficient of $\epsilon = 2.05 \times 10^4$ cm⁻¹ M⁻¹, therefore a solution containing 1 mg/ml has an absorbance of 0.9. The fraction of unbiotinylated CNTF in the preparation was evaluated by first boiling about 1 mg of the bCNTF in a buffer containing 50 mM Tris (pH 8.2) and 1% SDS, and then incubating the sample with an excess amount of streptavidin-agarose beads (SA-agar; Sigma) for 1 hour at room temperature. A parallel sample was prepared with SA-agar that has been saturated with excess biotin prior to addition of bCNTF. The beads are removed by centrifugation, and the supernatant fractions are analyzed for unbound CNTF by silver staining following SDS PAGE.

Typically greater than 90% of the CNTF becomes biotinylated by this procedure.

5 2.1.2 Preparation of bCNTF:SA-agar Complex and Extraction of
 CNTFR α from Plasma or Urine

 Biotinylated CNTF in PBS was incubated with SA-agar beads at a molar ratio of 1:3 (bCNTF : biotin binding capacity of the SA-agar) for 1 hour at room temperature. The beads were washed exhaustively with PBS, then resuspended in PBS to their original
10 volume. A 200 μ l aliquot of plasma (prepared in EDTA to prevent clotting) was diluted to 500 μ L in PBS containing 5mM EDTA, 1mM PMSF, 4.8×10^{-2} TIU aprotinin/ml and 0.1mg pepstatin A/ml, then incubated with 50 ml of the resuspended bCNTF-SA-agar complex for 2 hours at room temperature on a labquake mixer. The beads were
15 washed once with PBS, then the captured proteins were released by boiling in SDS PAGE sample buffer and analyzed by immunoblotting with a rabbit antibody (RG30) raised and immunopurified against a peptide corresponding to the N-terminus of human CNTFR α . Immunoreactive proteins were visualized with goat anti-rabbit Ig
20 conjugated to either alkaline phosphatase or 125 I. Use of the radioactive secondary reagent permitted quantitation of the reactive CNTFR α bands with the Fuji BioImager. Control experiments using recombinant CNTFR α released from fibroblasts with phospholipase C (which cleaves the GPI anchor to give a soluble protein) indicated
25 recoveries of 50-80% and a nearly linear response over a >10-fold concentration range (e.g. Figure 1, lanes 1 and 2).

 Urine samples were analyzed using a similar method on a larger scale. Quantitation of urine samples required normalization

to the urine level of creatinine, an indicator of the variable water content between individual urine samples.

2.2 Results

5 2.2.1 Analysis of CNTFR α in plasma after sciatic nerve crush

Analysis of plasma following bilateral sciatic nerve crush in the rat revealed a transient increase in the amount of CNTFR α in the blood (Figure 1). Various experiments revealed that the level of CNTFR α peaked between 30-48 hours after injury and generally fell
10 by 72-80 hours, lagging slightly behind the increase observed in the muscle mRNA level. Quantitation of CNTFR α in experiments with iodinated secondary reagents showed that this increase measured about 5-fold above the control level.

15 2.2.2 Analysis of CNTFR α in Urine from Control and Denervated Rats

Analysis of the urine from control and denervated rats revealed the same trend as above for plasma: normal animals had a
20 low or undetectable level of urine CNTFR α , which rose significantly by 48 hours after denervation. The migration of the CNTFR α in the gel was close to that of the phospholipase-cleaved standard, indicating that the GPI diacylglycerol had been cleaved, or that the entire GPI had been removed. The soluble CNTFR α appeared to be
25 functional as it retained the ability to bind CNTF.

2.2.3 Analysis of CNTFR α in urine from ALS patients

Given the striking increase in the level of CNTFR α in blood and urine upon denervation in rats, we tested whether humans suffering

from ALS, in whom progressive muscle denervation occurs, might also display increased CNTFR α levels. Urine from 4 patients with ALS was analyzed by the method outlined above, and compared to the amount of CNTFR α observed in 4 unaffected subjects(Figure 2)

5 Although all individuals excreted detectable amounts of CNTFR α in their urine, ALS patients exhibited elevated levels relative to the levels found in the unaffected subjects. (Figure 2, lanes 3-6).

10 Table 1 reveals that the ALS patients as a group had a 4.3-fold elevation in urine CNTFR α relative to the control group. A t-test of these values indicates the groups are statistically different at the level of $P < 0.02$. In contrast, the level of creatinine between the two groups was not statistically different.

15

Table 1. Quantitation of creatinine and CNTFR α in human urine

		Creatinine (mg/ml)	CNTFR α (ng/ml)	CNTFR α /Creatinine (ng/mg)
5				
	ALS #1	1.17	10.7	9.15
	ALS #2	1.80	13.2	7.33
	ALS #3	0.29	5.6	19.3
	ALS #4	0.77	14.9	19.4
10	Mean + S.D.	1.01 + 0.64		<u>13.8 + 6.5</u>
	Norm #1	0.16	0.5	3.2
	Norm #2	2.47	12.9	5.2
	Norm #3	1.38	4.8	3.5
	Norm #4	1.19	0.9	0.8
15	Mean + S.D.	1.29 + 0.95		<u>3.2 + 1.8</u>

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of detecting muscle denervation in a patient afflicted with such muscle denervation comprising:
 - a) obtaining from said patient a sample of a body fluid;
 - 5 b) reacting said sample with a material which specifically binds to ciliary neurotrophic factor receptor alpha protein (CNTFR α) which is present in solution in said sample to provide a complex of CNTFR α and said material;
 - c) determining a level of CNTFR α in said sample based on a measurement of said complex;
 - 10 d) comparing the level of CNTFR α determined in said sample to levels of soluble CNTFR α determined in samples of the body fluid of unaffected individuals, wherein an elevated level of CNTFR α in the sample is indicative of muscle denervation in the patient.
2. The method of claim 1 wherein said muscle denervation is associated with
 - 15 a motor neuron disease or disorder.
3. The method of claim 2 wherein said motor neuron disease is amyotrophic lateral sclerosis.
4. The method of claim 1 wherein said muscle denervation is associated with nerve injury.
- 20 5. The method of claim 1 wherein said body fluid is urine, blood or plasma.
6. The method of claim 1 wherein said material is an antibody specific for CNTFR α .

DATED: 5 August, 1996

25 PHILLIPS, ORMONDE & FITZPATRICK

Attorneys for:

REGENERON PHARMACEUTICALS INC



Fig. 1

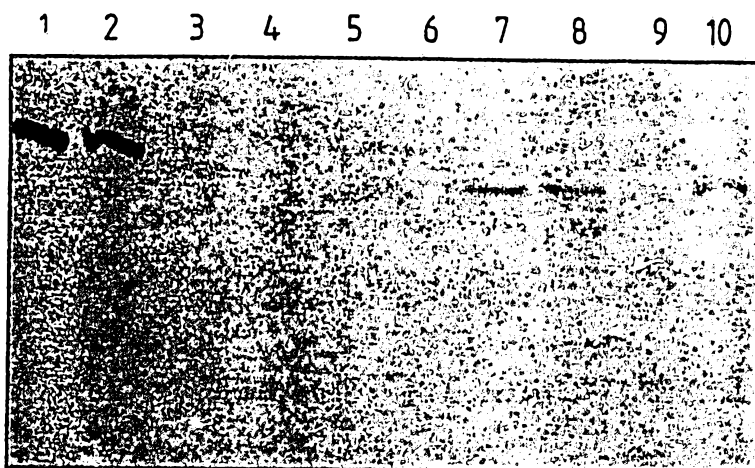
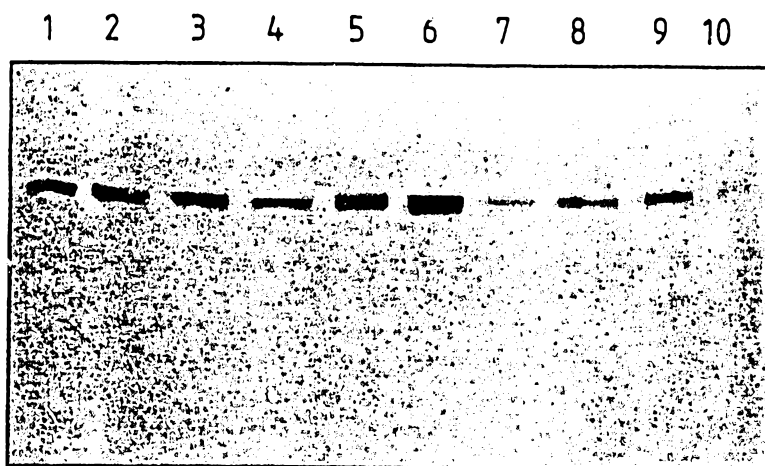


Fig. 2



INTERNATIONAL SEARCH REPORT

International Application No

PCT/US

8

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.Cl. 5 G01N33/68; G01N33/561		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 5	G01N ; C07K	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
P,X	SCIENCE vol. 259, no. 5102, 19 March 1993, LANCASTER, PA US pages 1736 - 1739 SAMUEL DAVIS ET AL. 'Released Form of CNTF Receptor alpha Component as a Soluble Mediator of CNTF Responses' see the whole document ---	1-19
X	WO,A,9 119 009 (REGENERON PHARMACEUTICALS; INC.) 12 December 1991 cited in the application see claims 29,32	1-19
A	see page 39, line 1 - page 47, line 17; claim 28; examples 8,9 ---	1-19
	-/--	
¹⁰ Special categories of cited documents : ^{"A"} document defining the general state of the art which is not considered to be of particular relevance ^{"E"} earlier document but published on or after the international filing date ^{"L"} document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) ^{"O"} document referring to an oral disclosure, use, exhibition or other means ^{"P"} document published prior to the international filing date but later than the priority date claimed ^{"T"} later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention ^{"X"} document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step ^{"Y"} document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. ^{"A"} document member of the same patent family		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search 23 1993		Date of Mailing of this International Search Report 13. 10. 93
International Searching Authority EUROPEAN PATENT OFFICE		Signature of Authorized Officer DÖPFER K.P.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	SCIENCE vol. 253, no. 5015, 5 July 1991, LANCASTER, PA US pages 59 - 63 SAMUEL DAVIS ET AL. 'The Receptor for Ciliary Neurotrophic Factor' ---	
T	NEUROSCIENCE RESEARCH vol. 15, no. 3, November 1992, NEW YORK NY US pages 151 - 161 TETSUO YAMAMORI AND AKINORI SARAI 'Coevolution of cytokine receptor families in the immune and nervous system' ---	
T	CURRENT OPINION IN CELL BIOLOGY vol. 5, no. 2, April 1993, LONDON GB pages 281 - 285 SAMUEL DAVIS AND GEORGE D. YANCOPOULOS 'The molecular biology of the CNTF receptor' -----	

US 9306528
SA 76912

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		CA-A- 2084075	02-12-91
		CN-A- 1061623	03-06-92
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